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SEMI-ANNUAL PROGRESS REPORT

Report Prepared By: Thomas F. Anderson Date: July 1, 1952

For Period January 1 to May 31, 1952

NR: 135-197

CONTRACT: N6-ori-168 Task Order II

ANNUAL RATE: $17,000

CONTRACTOR: The University of Pennsylvania

PRINCIPAL INVESTIGATOR: Thomas F. Anderson

Assistants: Dr. Catherine F. Rappaport (Full time; received Ph.D. in February)
Mr. Carl F. Oster, Jr. (Full time; student in College Collateral Course)
Mrs. Eva Dudley (One quarter time; dishwasher)
Mr. Robert L. Tyson (Full time during summers only; B. S. Yale 1952; starts at Columbia Medical School in Fall)

TITLE OF PROJECT: Physical, Chemical, and Biophysical Characterization of Viruses and Virus Systems.

Objectives:

A. To determine the nature and functional anatomy of virus particles.

B. To determine the interactions between bacteriophage virus particles which lead to infection of the cell and to the formation of daughter virus particles.

C. To promote naval applications of the knowledge gained, especially in the fields of medicine and physics.

Abstract of Results

A. Since start of project:

1) We have found that certain strains of the phages T4 and T6 require activation by an L-amino acid like tryptophan before they are adsorbed on their host cells. This requirement is inherited and in some cases seems to be met by the synthesis of adsorption cofactors by the host cells which thus make themselves susceptible to the virus's attack.

2) Gross artifacts in drying specimens for the electron microscope can be eliminated by warming the ambient liquid above its critical point and allowing the gas to escape at this temperature. In this way no phase boundary passes through the specimen. By means of this technique we have found that

a) The heads of the related phages T2, T4, and T6 appear

to have the shapes of hexagonal biprisms as does that of the unrelated phage T5. After the internal structure (DNA) has been removed by osmotic shock the empty head membranes tend to retain this shape.

b) The viruses T2, T4, T5, and T6 all of which have "tails" attach to host cells by their "tails."

3) Small "tailless" bacteriophages like T3 are resistant to sonic vibration so that virus multiplication can be studied by disrupting host cells during virus growth and studying their contents. In this way it was discovered that the infecting particles make their appearance only after 2/3 of the time required for lysis has been allowed for their development.

4) Neutralizing antiserum can apparently be stripped from T3 by sonic vibration to yield active T3 particles.

5) The large phages T2, T4, and T6 can be disintegrated by placing them in a concentrated solution of NaCl or some other solute and then rapidly diluting in water. If the dilution is made slowly the phages are not disintegrated; for this reason the phenomenon has been termed "osmotic shock." The virus particles in a given preparation are inhomogeneous with respect to osmotic shock, some being sensitive, others resistant. The proportion of resistant forms increases as the temperature is raised and decreases as the temperature is lowered. The rates of these transformations have been measured. The low temperature form is inactivated directly by 2.2M sucrose, but the high temperature form is not inactivated in the presence of Tween 60. It is supposed that these changes in properties of T6r are due to changes in the permeability of the head membranes of the virus particles.

B. <u>During current report period</u>:

1) An attempt was made to measure the permeability of the membranes of the heads of T-even phages by observing the rate of quenching of the fluorescence of the dye acriflavine as it penetrates the head and combines with the nucleic acid (DNA) within. It was found (a) that the dye combines rapidly with the DNA of shocked or heat-killed phage; (b) that the dye combines very slowly with the DNA of intact active phage below 60C but more rapidly in the presence of detergents; and (c) that the combination is reversed in the presence of ions such as H^+, Na^+, or Mg^{++} which presumably displace the dye from sites on the nucleic acid.

2) Dr. Rappaport has been studying adaptive enzyme formation in yeast as an example of the synthesis of specific biological material. She finds that in air the cells utilize glucose at low concentrations mainly through <u>respiration</u>, but that after

about two hours in air with high glucose concentrations the cells suddenly begin to ferment glucose as well. This increased fermentation reacts to K^+ and temperature in the same way as anaerobic fermentation but it reacts differently from the small fermentation which is observed initially in air. The hormone insulin accelerates the appearance of the anaerobic type of fermentation in air.

These results indicate that there are at least two different pathways for the utilization of glucose, an aerobic pathway and an alternate adaptive pathway. The latter pathway may be established when the aerobic system is blocked or saturated by the accumulation of some intermediate which is common to the two systems.

Plans for Future:

Immediate:

Dr. Rappaport will use Dr. Chance's spectrophotometric techniques to follow the formation of adaptive enzymes and investigate further the role of insulin in her system. Dr. Anderson will study further the nature of osmotic shock.

Long Range:

As we see it, our basic task is to learn the physics and chemistry involved in the specific reduplication of biological units. At present the bacterial viruses seem to be the best material for this study, but we shall be on the lookout for material which is even more favorable for the pursuit of this basic task. The formation of adaptive enzymes may be such a system.

REPORTS AND PUBLICATIONS (During Current Report Period).

Anderson, T. F. and Doermann, A. H., "Sonic reactivation of antiserum-neutralized bacteriophage T3", J. Bact., 63, 291-292 (1952).

Anderson, T. F. and Doermann, A. H., "The intracellular growth of bacteriophages. II. The growth of T3 studied by sonic disintegration and by T6-cyanide lysis of infected cells", J. Gen. Physiol., 35, 657-667 (1952).

Anderson, T. F., "Stereoscopic studies of cells and viruses in the electron microscope", The American Naturalist 84, 91-100 (1952).